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(54) Title: **NOVEL CHEMOKINE FOR MOBILIZING STEM CELLS**

(57) Abstract

Novel chemokines for mobilizing stem cells are provided. Methods of mobilizing stem cells are also provided.

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NOVEL CHEMOKINE FOR MOBILIZING STEM CELLS

Background of the Invention

5 Hematopoietic cells have very important roles in a number of different processes in the body. For example, leukocytic hematopoietic cells are important in maintaining the body's defenses against disease; monocytes, macrophages and lymphocytes are involved in potentiating the body's responses to infection and tumors, while granulocytes are involved in overcoming infection, parasites and tumors. Platelets, another hematopoietic cell, form an important element in the
10 hemostatic mechanism through initiating thrombus formation by their adhesion to each other and to damaged surfaces, and by the release of factors which assist in the formation of the fibrin clot. Erythrocytes are mainly involved in the transport of oxygen.

15 All of these blood cells are derived from a single progenitor cell called the hematopoietic stem cell. Stem cells are both pluripotent, in that they give rise to all different cell types, and capable of self renewal. Hematopoietic stem cells make up only a small percentage of bone marrow cells and are normally quiescent. However, when stimulated to divide, these stem cells produce a differentiated daughter cell with great proliferative potential. Sequential rounds of division and differentiation give
20 rise to an enormous amplification of cell numbers which is necessary for the production of mature blood cells. This process of division and differentiation is subject to regulation at many levels to control cell production.

Numerous studies have led to the definition of functions of several hematopoietic regulatory messengers. These biomolecules have been characterized
25 as stimulatory, e.g., Colony Stimulating Factors (CSFs) and interleukins (IL-1, IL-3, IL-5 and IL-9); inhibitory, e.g., transforming growth factor- β (TGF- β), interferon, prostaglandin E, tumor necrosis factor, macrophage inflammatory protein-1 (MIP-1), lactoferrin, acidic isoferitins, AcSKDP, and pEEDCK (a synthetic HP5B monomer); or enhancing, e.g., TGF- β , IL-6, IL-4, IL-9, IL-11, MIP-1, MIP-2, leukemia
30 inhibitory factor and *Steel* factor. Pelus et al. *Experimental Hematology* 1994, 22:239-247. Stimulatory biomolecules have been found to promote division of

particular cell lineages. For example, G-CSF derives neutrophil production, while erythropoietin promotes formation of erythrocytes.

A number of these biomolecules and additional agents have been found to induce the mobilization of hematopoietic stem cells.

5 A single injection of IL-8 has been shown to induce mobilization of pluripotent stem cells that are able to provide permanent reconstitution of myeloid cells and of T and B lymphocytes. Laterveer et al. *Blood* 1995, 85(8):2269-2275. IL-8 belongs to a family of pro-inflammatory molecules called chemokines. This family has been divided into two subfamilies, the CXC and CC chemokines, based on
10 whether the first two cysteine residues in a conserved motif are adjacent to each other or are separated by an intervening residue. In general, CXC, which include IL-8, melanoma growth-stimulating activity (MGSA) and platelet factor 4 (PF4), are potent chemoattractants and activators of neutrophils but not monocytes. In contrast, CC chemokines, which include RANTES, monocyte chemotactic protein 1
15 (MCP-1) and MIP-1, are chemoattractants for monocytes but not neutrophils.

Stem cell inhibitors (SCIs) such as the CC chemokines, murine and human MIP-1 α (LD78), have also been shown to enhance the release and mobilization of cells into the peripheral blood. WO 94/28916; Simm et al. *Blood* 1994, 84:2937.

Increased mobilization of stem cells in patients treated with sequentially
20 administered interleukin-3 and GM-CSF compared with GM-CSF alone has been reported by Brugger et al. *Blood* 1992, 79:1193-1200. In addition, it has been shown that the absolute number of peripheral blood progenitor cells can be expanded *in vitro* by culture in a cocktail of cytokines, usually including SCF, IL-3, and either IL-6 or IL-1. Bodine, D. *Experimental Hematology* 1995, 23:293-295.

25 SK&F 107647, a hematoregulatory agent containing an ethylene bridge in place of the cysteine bridge of HP5B, has been demonstrated to be a potent stimulator of *in vitro* myelopoiesis. Pelus et al. *Experimental Hematology* 1994, 22:239-247. Injection of SK&F 107647 in normal mice resulted in a two- to six-fold increase in serum colony-stimulating activity. Administration of this agent over 4
30 days resulted in significant increases in the number of granulocyte-macrophage,

erythroid, and multipotential progenitor cells, as well as stimulating their cell cycle rates.

It has also been found that pretreatment with stem cell stimulating factor such as G-CSF can expand the pool of progenitor cells susceptible for mobilization by these agents, further increasing their mobilizing effect. For example, the combination of MIP-1 α with G-CSF was found to increase white cell count in the blood as compared to G-CSF alone. Simm et al. *Blood* 1994, 84:2937. Co-administration of SCI with G-CSF caused the enhanced mobilization of a number of cell types including neutrophils, monocytes, eosinophils, lymphocytes and basophils. WO 94/28916. Administration of G-CSF alone had no effect on the release of eosinophils or basophils after 2 days of administration. Similar effects were observed when other agents such as GM-CSF, f-MET-Leu-Phe or IL-8 were coadministered with SCIs.

New chemokines have now been identified which also mobilize stem cells in an animal. These chemokines can be administered alone, or in combination with a colony stimulating factor or hemoregulatory agent to enhance mobilization of stem cells.

Summary of the Invention

An object of the present invention is to provide novel chemokines for the mobilization of stem cells in an animal.

Another object of the invention is to provide a method of mobilizing stem cells.

Brief Description of the Drawings

Figure 1 shows the sequence and alignment of the novel chemokines with known chemokines.

Detailed Description of the Invention

In recent years, the availability of recombinant cytokines and the use of hematopoietic stem cell support have resulted in the widespread application of high-dose chemotherapy regimens designed to improve the success of cancer therapy.

Despite significant advances, however, delayed recovery of hematopoiesis remains an important source of morbidity and mortality for patients treated with this approach. Since their discovery over 20 years ago, peripheral blood hematopoietic progenitor cells (PBPCs) have been increasingly used to supplement and even replace bone marrow as the source of hematopoietic support in a variety of situations.

Purified populations of cells are increasingly being used therapeutically and it would therefore be advantageous to be able to increase the number of circulating blood cells. It is useful to be able to harvest hematopoietic cells prior to chemotherapy or radiotherapy, thus, protecting them from harmful effects of this therapy; after therapy, the cells can be returned to the patient. It would therefore be highly beneficial to provide an agent which promoted the release and mobilization of a number of hematopoietic cells. Such an agent would be useful for enhancing the response to infection.

Peripheral blood cell transplantation is an important procedure in the treatment of cancer patients with high dose chemotherapy. In such treatment, patients are treated to induce clinical remission of their cancer, then during the remission, successive treatment with CSF, for example, by priming with cyclophosphamide then administration of G-CSF, causes eventual mobilization of cells from the bone marrow to the peripheral circulation for harvesting of leukophoresed blood; then the patient is given high dose chemotherapy or radiotherapy and the resultant bone marrow failure is compensated for by infusion of the stored blood or cells collected previously. This procedure may be modified by the omission of the initial induction of remission, and whole blood may be collected rather than leukophoresed blood. The mobilization effects of the present invention makes it a candidate both to replace CSFs in such cancer treatment regimes, and also to complement the mobilization effects of CSFs in combined treatments.

The two subfamilies of chemokines (CXC and CC) are ever expanding and presumably the individual members have similar, if slightly divergent, functions. The chemokines disclosed in the present invention are new members of the CC subfamily and are structurally similar to MCP-1, MCP-3, hRANTES, mMIP-1 α , and mMIP-1 β (Figure 1). The effect of these chemokines in inducing leukophilia will find clinical

and veterinary application in all utilities where the raising of hematopoietic cell levels is important. For example, a chemokine of the present invention can be used to enhance immune responses against chronic infections, particularly parasitic and bacterial infections. It may also have a role in promoting wound healing.

5 The chemoattractant activity of these chemokines can be boosted by pretreatment with a colony stimulating factor such as G-CSF or GM-CSF. Alternatively, the hematoregulatory peptides SK&F 107647 (currently in clinical trials), FLT-3 ligand (Immunex) or any other G-CSF mimetics (peptide and non-peptide) may be used. These stimulants may have an even more dramatic effect on
10 these novel chemokines than on those already known due to their slight structural differences. For example, CKB-6 in combination with G-CSF was effective as a mobilizing factor. As known in the art, these peptides are useful in stimulating myelopoiesis in patients suffering from reduced myelopoietic activity, including bone marrow damage, agranulocytosis and aplastic anemia. Also included are patients
15 who have depressed bone marrow function due to immunosuppressive treatment to suppress tissue reactions (i.e., bone marrow transplant surgery). They may also be used to promote more rapid regeneration of bone marrow after cytostatic chemotherapy and radiation therapy for neoplastic and viral diseases. There may also be a value where patients have serious infections due to a lack of immune response
20 following bone marrow failure.

 The hematopoietic stem cells released and harvested in the manner described above may be useful for subsequent *in vitro* and *ex vivo* manipulations to deliver gene products in gene therapy. Another embodiment is co-administration with cytotoxic drugs.

25 The following examples are provided for illustrative purposes only and are not intended to limit the invention.

EXAMPLES

Example 1: Mobilization Assay for Novel Chemokines as Single Agents

30 A panel of novel chemokines will be tested as individual stem cell mobilization agents in BDF 1 mice. These chemokines include, but should not be

limited to: Ck β -1, Ck β -4, Ck β -6, Ck β -7, Ck β -8, Ck β -9, Ck β -10, Ck β -11, Ck β -12, Ck β -13, and Ck α -1. Each agent will be assayed in concentrations of 50, 10, and 2 μ g/mouse and administered via SC, IM, or a PO route. The kinetics of chemokine mobilization of stem cells will be monitored in 15 minute intervals over a period of 60 minutes by collecting blood samples from the mice by cardiac puncture. The mobilized stem cells will be collected by a density gradient (Lympholyte M). Cells are washed then frozen for future usage. The mobilization profile of the blood differentials will be assessed using a Technicon HI hematology analyzer. Mobilization of inflammatory cells such as PMN's, eosinophils, and basophils will be taken into account when evaluating the overall potential inflammatory profile. The chemokine IL-8, which mobilizes hematopoietic stem cells as a single factor, will be included in these studies as a positive control.

Example 2: Mobilization Assay for Novel Chemokines in Combination with Hematostimulants

In these studies, hematostimulants will be assayed in combination with the aforementioned chemokines as mobilization factors. These agents include: G-CSF, GM-CSF, SK&F 107647, and FLT-3 ligand. However, any G-CSF mimetic (hematostimulants which are not colony stimulating factors like G-CSF or GMCSF, but have hematopoietic activity) may be used. In combination studies, G-CSF will be administered IP to mice four days prior to the novel chemokines. As in Example 1, the dose of chemokine and time of blood collection will be varied. Combination studies with hematostimulant pre-treatment will utilize MIP-1 α as the positive control.

Example 3: CFU Assay

Blood samples collected during the mobilization phase will be assessed for colony forming units (CFU-GM) at days 7 and 14. Cells are adjusted to 2×10^6 cells/ml in McCoy's medium with 15 x FBS serum. A single layer agar system utilizing the following is used: McCoy's medium enriched with nutrients (NaHCO_3 ,

pyruvate, amino acids and vitamins); 0.3% Bacto agar. To this is added cells from the blood samples (final concentration = 2×10^5 cells/ml). The agar plates are incubated at 37°C, 5% CO₂ for 7 days. Colonies of proliferating cells (CFU-GM) are counted utilizing a microscope. In addition, early hematopoietic high proliferative potential (HPP) progenitors, will be counted in the day 14 CFU cultures.

5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SmithKline Beecham Corporation and Human Genome Sciences, Inc.

(ii) TITLE OF INVENTION: Novel Chemokine for Mobilizing Stem Cells

(iii) NUMBER OF SEQUENCES: 19

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(E) COUNTRY: USA

(F) ZIP: 19406-0939

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(C) REFERENCE/DOCKET NUMBER: P50382

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

THR	LYS	THR	GLU	SER	SER	SER	ARG	GLY	PRO	TYR	HIS	PRO	SER	GLU
1				5				10					15	
CYS	CYS	PHE	THR	TYR	THR	THR	TYR	LYS	ILE	PRO	ARG	GLN	ARG	ILE
				20				25					30	
MET	ASP	TYR	TYR	GLU	THR	ASN	SER	GLN	CYS	SER	LYS	PRO	GLY	ILE
				35				40					45	
VAL	PHE	ILE	THR	XAA	ARG	GLY	HIS	SER	VAL	CYS	THR	ASN	PRO	SER
				50				55					60	
ASP	LYS	TRP	VAL	GLN	ASP	TYR	ILE	LYS	ASP	MET	LYS			
				65				70						

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ALA	SER	ASN	PHE	ASP	CYS	CYS	LEU	GLY	TYR	THR	ASP	ARG	ILE	LEU
1				5				10					15	
HIS	PRO	LYS	PHE	ILE	VAL	GLY	PHE	THR	ARG	GLN	LEU	ALA	ASN	ASN
				20				25					30	
GLY	CYS	ASP	ILE	ASN	ALA	ILE	ILE	PHE	HIS	THR	LYS	LYS	LYS	LEU
				35				40					45	
SER	VAL	CYS	ALA	ASN	PRO	LYS	GLN	THR	TRP	VAL	LYS	TYR	ILE	VAL
				50				55					60	
ARG	LEU	LEU	SER	LYS	LYS	VAL	LYS	ASN	MET					
				65				70						

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

VAL	VAL	ILE	PRO	SER	PRO	CYS	CYS	MET	PHE	PHE	VAL	SER	LYS	ARG
1						5					10			15
ILE	PRO	GLU	ASN	ARG	VAL	VAL	SER	TYR	GLN	LEU	SER	SER	ARG	SER
					20					25				30
THR	CYS	LEU	LYS	GLY	GLY	VAL	ILE	PHE	THR	THR	LYS	LYS	GLY	GLN
					35					40				45
GLN	PHE	CYS	GLY	ASP	PRO	LYS	GLN	GLU	TRP	VAL	GLN	ARG	TYR	MET
					50					55				60
LYS	ASN	LEU	ASP	ALA	LYS	GLN	LYS	LYS	ALA					
					65					70				

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ALA	GLN	VAL	GLY	THR	ASN	LYS	GLU	LEU	CYS	CYS	LEU	VAL	TYR	THR
1						5					10			15
SER	TRP	GLN	ILE	PRO	GLN	LYS	PHE	ILE	VAL	ASP	TYR	SER	GLU	THR
					20					25				30
SER	PRO	GLN	CYS	PRO	LYS	PRO	GLY	VAL	ILE	LEU	LEU	THR	LYS	ARG
					35					40				45
GLY	ARG	GLN	ILE	CYS	ALA	ASP	PRO	ASN	LYS	LYS	TRP	VAL	GLN	LYS
					50					55				60

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GLU	ASN	PRO	VAL	LEU	LEU	ASP	ARG	PHE	HIS	ALA	THR	SER	ALA	ASP
1					5					10				15
CYS	CYS	ILE	SER	TYR	THR	PRO	ARG	SER	ILE	PRO	CYS	SER	LEU	LEU
					20					25				30

```

GLU SER TYR PHE GLU THR ASN SER GLU CYS SER LYS PRO GLY VAL
      35                      40                      45
ILE PHE LEU THR LYS LYS GLY ARG ARG PHE CYS ALA ASN PRO SER
      50                      55                      60
ASP LYS GLN VAL GLN VAL CYS MET ARG MET LEU LYS LEU ASP THR
      65                      70                      75
ARG ILE LYS THR ARG LYS ASN
      80

```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```

SER ASP ALA GLY GLY ALA GLN ASP CYS CYS LEU LYS TYR SER GLN
1      5                      10                      15
ARG LYS ILE PRO ALA LYS VAL VAL ARG SER TYR ARG LYS GLN GLU
      20                      25                      30
PRO SER LEU GLY CYS SER ILE PRO ALA ILE LEU PHE LEU PRO ARG
      35                      40                      45
LYS ARG SER GLN ALA GLU LEU CYS ALA ASP PRO LYS GLU LEU TRP
      50                      55                      60
VAL GLN GLN LEU MET GLN HIS LEU ASP LYS THR PRO SER PRO GLN
      65                      70                      75
LYS PRO ALA GLN

```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 82
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```

PHE ASN PRO GLN GLY LEU ALA GLN PRO ASP ALA LEU ASN VAL PRO
1      5                      10                      15
SER THR CYS CYS PHE THR PHE SER SER LYS LYS ILE SER LEU GLN
      20                      25                      30
ARG LEU LYS SER TYR VAL ILE THR THR SER ARG CYS PRO GLN LYS
      35                      40                      45

```

ALA VAL ILE PHE ARG THR LYS LEU GLY LYS GLU ILE CYS ALA ASP
 50 55 60
 PRO LYS GLU LYS TRP VAL GLN ASN TYR MET LYS HIS LEU GLY ARG
 65 70 75
 LYS ALA HIS THR LEU LYS THR
 80

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

PRO ALA PRO THR LEU SER GLY THR ASN ASP ALA GLU ASP CYS CYS
 1 5 10 15
 LEU SER VAL THR GLN LYS PRO ILE PRO GLY TYR ILE VAL ARG ASN
 20 25 30
 PHE HIS TYR LEU LEU ILE LYS ASP GLY CYS ARG VAL PRO ALA VAL
 35 40 45
 VAL PHE THR THR LEU ARG GLY ARG GLN LEU CYS ALA PRO PRO ASP
 50 55 60
 GLN PRO TRP VAL GLU ARG ILE ILE GLN ARG LEU GLN ARG THR SER
 65 70 75
 ALA LYS MET LYS ARG ARG SER SER
 80

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 82
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ARG SER GLN PRO LYS VAL PRO GLU TRP VAL ASN THR PRO SER THR
 1 5 10 15
 CYS CYS LEU LYS TYR TYR GLU LYS VAL LEU PRO ARG ARG LEU VAL
 20 25 30
 VAL GLY TYR ARG LYS ALA LEU ASN CYS HIS LEU PRO ALA ILE ILE
 35 40 45

PHE VAL THR LYS ARG ASN ARG GLU VAL CYS THR ASN PRO ASN ASP
 50 55 60
 ASP TRP VAL GLN GLU TYR ILE LYS ASP PRO ASN LEU PRO LEU LEU
 65 70 75
 PRO THR ARG ASN LEU SER THR
 80

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

PRO TYR GLY ALA ASN MET GLU ASP SER VAL CYS CYS ARG ASP TYR
 1 5 10 15
 VAL ARG TYR ARG LEU PRO LEU ARG VAL VAL LYS HIS PHE TYR TRP
 20 25 30
 THR SER ASP SER CYS PRO ARG PRO GLY VAL VAL LEU LEU THR PHE
 35 40 45
 ARG ASP LYS GLU ILE CYS ALA ASP PRO ARG VAL PRO TRP VAL LYS
 50 55 60
 MET ILE LEU ASN LYS LEU SER GLN
 65

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ALA SER PRO TYR SER SER ASP THR THR PRO CYS CYS PHE ALA TYR
 1 5 10 15
 ILE ALA ARG PRO LEU PRO ARG ALA HIS ILE LYS GLU TYR PHE TYR
 20 25 30

THR SER GLY LYS CYS SER ASN PRO ALA VAL VAL PHE VAL THR ARG
 35 40 45
 LYS ASN ARG GLN VAL CYS ALA ASN PRO GLU LYS LYS TRP VAL ARG
 50 55 60
 GLU TYR ILE ASN SER LEU GLU MET SER
 65

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

ALA PRO TYR GLY ALA ASP THR PRO THR ALA CYS CYS PHE SER TYR
 1 5 10 15
 SER ARG LYS ILE PRO ARG GLN PHE ILE VAL ASP TYR PHE GLU THR
 20 25 30
 SER SER LEU CYS SER GLN PRO GLY VAL ILE PHE LEU THR LYS ARG
 35 40 45
 ASN ARG GLN ILE CYS ALA ASP SER LYS GLU THR TRP VAL GLN GLU
 50 55 60
 TYR ILE THR ASP LEU GLU LEU ASN ALA
 65

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

[illegible]

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 78
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

[illegible]

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

SER	PRO	GLN	GLY	LEU	ALA	GLN	PRO	VAL	GLY	ILE	ASN	THR	SER	THR
1				5					10					15
THR	CYS	CYS	TYR	ARG	PHE	ILE	ASN	LYS	LYS	ILE	PRO	LYS	GLN	ARG
				20					25					30
LEU	GLU	SER	TYR	ARG	ARG	THR	THR	SER	SER	HIS	CYS	PRO	ARG	GLU
				35					40					45
ALA	VAL	ILE	PHE	LYS	THR	LYS	LEU	ASP	LYS	GLU	ILE	CYS	ALA	ASP
				50					55					60
PRO	THR	GLN	LYS	TRP	VAL	GLN	ASP	PHE	MET	LYS	HIS	LEU	ASP	LYS
				65					70					75
LYS	THR	GLN	THR	PRO	LYS	LEU								
				80										

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

SER	ALA	LYS	GLU	LEU	ARG	CYS	GLN	CYS	ILE	LYS	THR	TYR	SER	LYS
1				5					10					15
PRO	PHE	HIS	PRO	LYS	PHE	ILE	LYS	GLU	LEU	ARG	VAL	ILE	GLU	SER
				20					25					30
GLY	PRO	HIS	CYS	ALA	ASN	THR	GLU	ILE	ILE	VAL	LYS	LEU	SER	ASP
				35					40					45
GLY	ARG	GLU	LEU	CYS	LEU	ASP	PRO	LYS	GLU	ASN	TRP	VAL	GLN	ARG
				50					55					60
VAL	VAL	GLU	LYS	PHE	LEU	LYS	ARG	ALA	GLU	ASN	SER			
				65					70					

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ALA	GLU	LEU	ARG	CYS	MET	CYS	ILE	LYS	THR	THR	SER	GLY	ILE	HIS
1				5					10					15
PRO	LYS	ASN	ILE	GLN	SER	LEU	GLU	VAL	VAL	ILE	GLY	LYS	GLY	THR
				20					25					30
HIS	CYS	ASN	GLN	VAL	GLU	VAL	ILE	ALA	THR	LEU	LYS	ASP	GLY	ARG
				35					40					45
LYS	ILE	CYS	LEU	ASP	PRO	ASP	ALA	PRO	ARG	ILE	LYS	LYS	ILE	VAL
				50					55					60
GLN	LYS	LYS	LEU	ALA	GLY	ASP	GLU	SER	ALA	ASP				
				65					70					

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GLU	ALA	GLU	GLU	ASP	GLY	ASP	LEU	GLN	CYS	LEU	CYS	VAL	LYS	THR
1				5					10					15
THR	SER	GLN	VAL	ARG	PRO	ARG	HIS	ILE	THR	SER	LEU	GLU	VAL	ILE
				20					25					30
LYS	ALA	GLY	PRO	HIS	CYS	PRO	THR	ALA	GLN	LEU	ILE	ALA	THR	LEU
				35					40					45

LYS ASN GLY ARG LYS ILE CYS LEU ASP LEU GLN ALA PRO LEU TYR
 50 55 60
 LYS LYS ILE LEU LYS LYS LEU GLU SER
 65

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

VAL LEU GLU VAL TYR TYR THR SER LEU ARG CYS ARG CYS VAL GLN
 1 5 10 15
 GLU SER SER VAL PHE ILE PRO ARG ARG PHE ILE ASP ARG ILE GLN
 20 25 30
 ILE LEU PRO ARG GLY ASN GLY CYS PRO ARG LYS GLU ILE ILE VAL
 35 40 45
 TRP LYS LYS ASN LYS SER ILE VAL CYS VAL ASP PRO GLN ALA GLU
 50 55 60
 TRP ILE GLN ARG MET MET GLU VAL LEU ARG LYS ARG
 65 70

What is claimed is:

1. A chemokine comprising SEQ ID NO: 1 capable of mobilizing stem cells.
2. A chemokine comprising SEQ ID NO: 2 capable of mobilizing stem cells.
3. A chemokine comprising SEQ ID NO: 3 capable of mobilizing stem cells.
4. A chemokine comprising SEQ ID NO: 4 capable of mobilizing stem cells.
5. A chemokine comprising SEQ ID NO: 5 capable of mobilizing stem cells.
6. A chemokine comprising SEQ ID NO: 6 capable of mobilizing stem cells.
7. A chemokine comprising SEQ ID NO: 7 capable of mobilizing stem cells.
8. A chemokine comprising SEQ ID NO: 8 capable of mobilizing stem cells.
9. A chemokine comprising SEQ ID NO: 9 capable of mobilizing stem cells.
10. A chemokine comprising SEQ ID NO: 10 capable of mobilizing stem cells.
11. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 1.
12. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 2.
13. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 3.
14. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 4.
15. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 5.
16. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 6.
17. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 7.
18. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 8.
19. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 9.

20. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 10.

21. The method of claims 11-20 further comprising administering a colony stimulating factor.

22. The method of claims 11-20 further comprising administering a hematoregulatory agent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16959

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/52; A61K 38/19
US CL :530/434; 424/85.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/434; 424/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

search terms: chemokine, mip

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	WO 95 18228 A1 (FORSSMANN) 06 July 1995 (06.07.95) Claim 1, SEQ ID NO:6	1,11 ----- 21,22 ----- 2-10,12-20
X -- Y -- A	WO 95/17092 A1 (HUMAN GENOME SCIENCES, INC.) 29 June 1995 (29.06.95) Claims 10, 12 and 48, Figures 1, 2 and 8.	1,4,5,11, 14,15 ----- 21,22 ----- 2, 3, 6 10,12,13,16-20

☒ X

Further documents are listed in the continuation of Box C.

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See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* Z	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

26 DECEMBER 1996

Date of mailing of the international search report

03 FEB 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16959

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	Brugger et al. Mobilization of peripheral blood progenitor cells by sequential administration of Interleukin-3 and granulocyte-macrophage colony-stimulating factor following polychemotherapy with etoposide, ifosfamide, and cisplatin. Blood. 01 March 1992, Vol. 79, No. 5, pages 1193-1200, see entire document.	21-22
A	Horuk. R. Molecular properties of the chemokine receptor family. TiPS. May 1994, Vol. 15, pages 159-165.	1-22
Y	Laterveer et al. Interleukin-8 induces rapid mobilization of hematopoietic stem cells with radioprotective capacity and long-term myelolymphoid repopulating ability. Blood. 15 April 1995, Vol. 85, No. 8, pages 2269-2275, see entire document.	21-22
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A		1-20

